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Our goal was to develop herpes vectors to treat prostate cancer in order to acquire the necessary data to advance toward clinical trials. We have studied G207, a multiple-mutated conditionally-replicating herpes simplex virus with deletions of both copies of gamma$_1$ 34.5 genes and a lacZ insertion disabling the ICP6 gene. We demonstrated that G207 is: 1, highly cytolytic in vitro to four of four human prostate cell lines tested in vitro; 2, effective in treating and capable of curing two different human prostate cancers grown in athymic mice; 3, effective in vivo on both hormone sensitive and on hormone refractory prostate cancers; 4, effective in vivo in human prostate cancer that is recurrent after radiation therapy. Current studies include the use of a syngeneic system for study of these effects and studies of potential toxicity of this form of therapy in order to progress to human trial.

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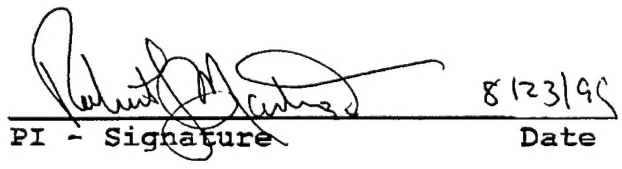

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DAMD17-98-1-8490
HERPES VIRUS THERAPY OF PROSTATE CANCER
Robert L. Martuza, P. I.

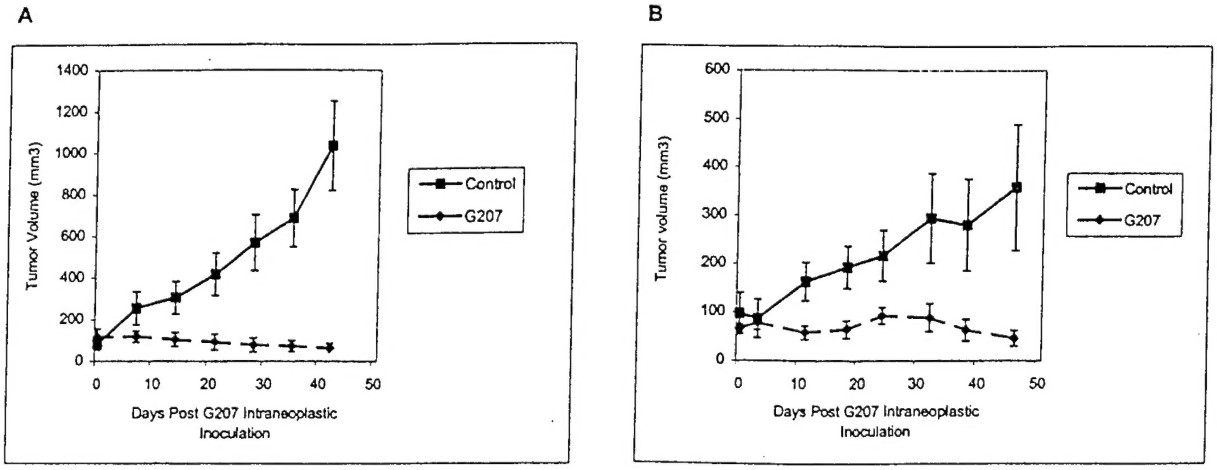
INTRODUCTION: As mentioned in our initial proposal , "our goal was to be able to use herpes vectors to treat local and metastatic prostate cancer and to advance toward clinical trials". Our initial goal was to develop G207 as a local therapy of recurrent prostate cancer in order to advance it into clinical trial. Our second goal was to further develop herpes vectors for use in metastatic prostate cancer. We have made significant progress in both of these areas in the first year of this grant.

BODY: G207 is a multiple-mutated conditionally-replicating herpes simplex virus with deletions of both copies of gamma$_1$ 34.5 genes [1] and a lacZ insertion disabling the ICP6 gene [2]. These confer upon G207 the following properties important to taking this into clinical trial for prostate cancer: 1) G207 is able to replicate in multiple types of human cancer cells and cause cancer cell death; 2) The presence of multiple widely-spaced mutations makes reversion to wild-type exceedingly unlikely and has not been seen through multiple passages. Moreover, even if reversion occurs at one locus, the vector remains markedly attenuated for neurotoxicity by virtue of the other mutations; 3) G207 has been extensively tested in Balb/c mice by intracerebral, intraventricular, and intravenous inoculation and in exquisitely sensitive subhuman primates (aotus) intracerebrally and shown to be nontoxic even after direct inoculation in brain at titers in excess of 10^9 pfu (plaque forming units of virus); 4) G207 is hypersensitive to acyclovir or ganciclovir. Thus it can be treated if unwanted replication ensues. This is not possible with adeno- or retro-viral vectors and is an important safety feature of G207; 5) Gamma$_1$ 34.5 deletion mutants have been shown to be very inefficient at reactivation (Roizman, pers. comm.); 6) G207 does not cause reactivation of previously existing wild-type HSV-1 in the brain. G207 can grow within and kill cancer cells without toxicity to normal cells including neural tissue. Efficacy of G207 has been demonstrated in models of nervous system malignancy (malignant glioma[3] and meningioma[4]) as well as in non-nervous system malignancy (human breast cancer[5] and squamous cell carcinoma [6]). In our initial grant proposal, we demonstrated that four of four human prostate cell lines tested in vitro are sensitive to G207 and three of four human prostate cell lines tested are ten times more sensitive in vitro than these other tumor cells.

PROGRESS THIS YEAR:

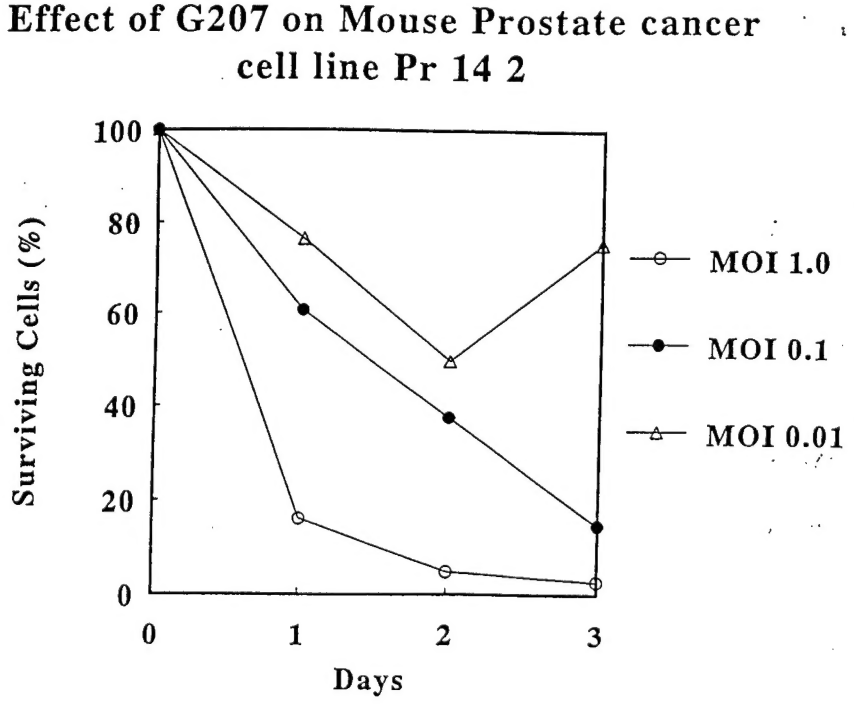
HYPOTHESIS 1. When inoculated intraneoplastically into a murine prostate cancer in an immune-competent syngeneic transgenic mouse, G207 will induce a cell-mediated anti-tumor response in both the primary inoculated tumor as well as in distant metastases, and will prevent or delay the development of prostate cancer ***in situ***:

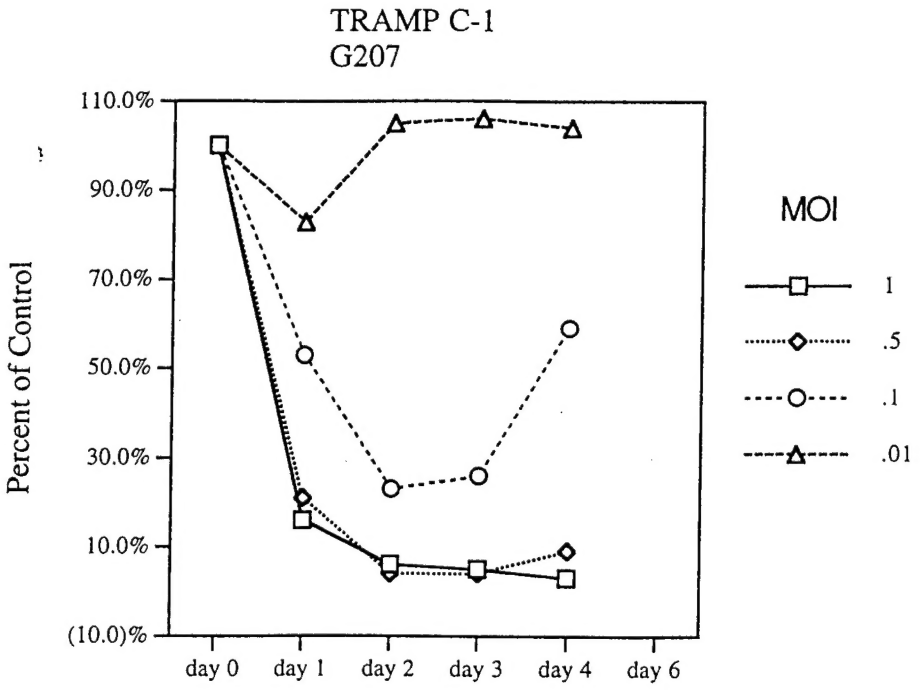
Towards the initial goal of developing a treatment of local prostate cancer (in situ and locally recurrent), we have now demonstrated that G207 causes tumor cell death, growth inhibition, and cures of human prostate cancers (LnCaP) implanted in athymic mice. We have further confirmed this with a second tumor type (DU-145). The following figures demonstrates inhibition and cure of LnCap and DU145 human prostate tumors following intratumoral inoculation of G207 in athymic mice:



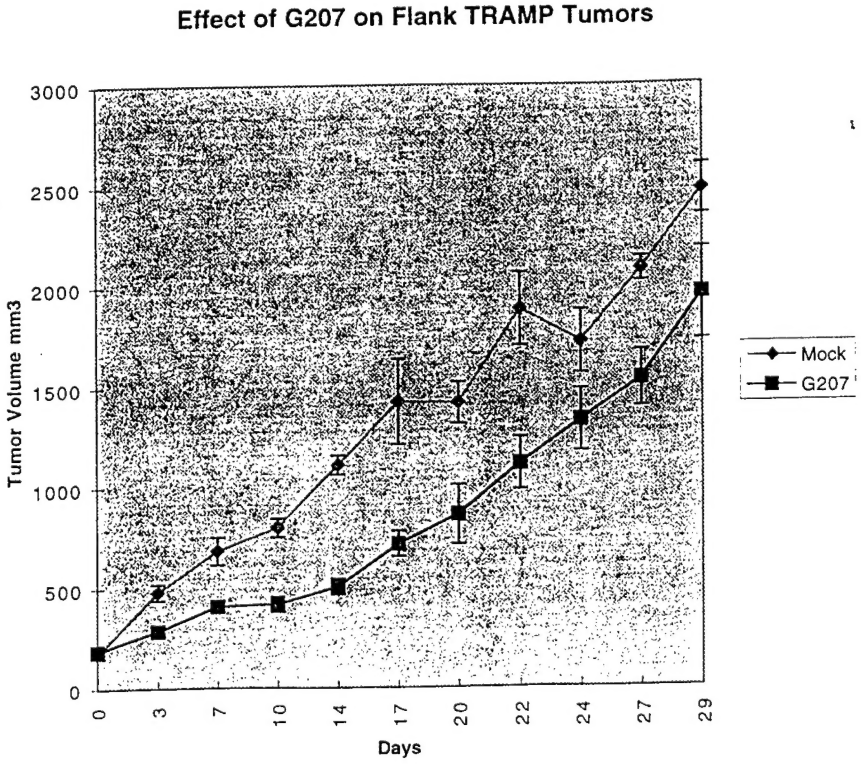
The effect of intraneoplatic injection of G207 on subcutaneous human prostate tumor growth. (A) LNCaP and (B) DU-145 subcutaneous tumors were established in BALB/c *nu/nu* mice. When the mean volume of LNCaP tumors (n = 8) and DU-145 tumors (n = 1b) was 99 mm^3 (range, 28–224 mm^3) and 80 mm^3 (range, 26–350 mm^3), respectively, tumors received a single intraneoplastic injection of G207 (2×10^7 PFU) or virus buffer (day 0). G207-treated tumors showed a reduction in volume, whereas buffer-treated tumors continued to grow (LNCaP, $p < 0.05$ versus controls on day 42; DU-145, $p < 0.01$ versus controls on day 46). Complete eradication of 25 and 22% of the LNCaP and DU-145 tumors, respectively, was noted. (Bars represent mean tumor volume ± the standard error of the mean.)

Our attempts to develop a syngeneic murine model for study have realized one of the first pitfalls we had mentioned in the grant application. The following figures demonstrate that while G207 can kill rodent prostate cancer cells in vitro, the efficacy is much less than with human prostate cancer cells as is shown both with PR-14-2 and with the TRAMP cells lines below[7,8]. A much higher MOI is required.





We further explored the TRAMP system in vivo and note only a modest tumor inhibition from intratumoral G207. The next figure demonstrates the effect of G207 on TRAMP-derived tumors. The effect is modest when compared to the human tumors in figures above.



This is not totally surprising, since as mentioned in the initial application, herpes cytolytic infection of rodent cells is known to be far less efficient than human cells. Nonetheless, it has delayed our work to some extent, however, this still may be a useful model to explore synergistic effects of radiation in that G207 is only partially effective. For tumors in which G207 is extremely effective it can be difficult to show an added effect of another therapy such as radiation for example.

TOXICITY OF INTRAPROSTATIC G207: We have begun our animal toxicity studies and have initial data suggesting that intraprostatic inoculation of G207 causes no nerve damage and is safe to the animal. We are now finishing the detailed analysis of tissues and we hope to complete this before the 30 months planned in the initial Statement of Work, and will include details in a future annual report. From the preliminary studies our expectation is that G207 can be used as a "nerve-sparing" method of treatment of locally recurrent prostate cancer, for example, in patients who have initially been treated with radiation and now have progressive local disease with a rising PSA.

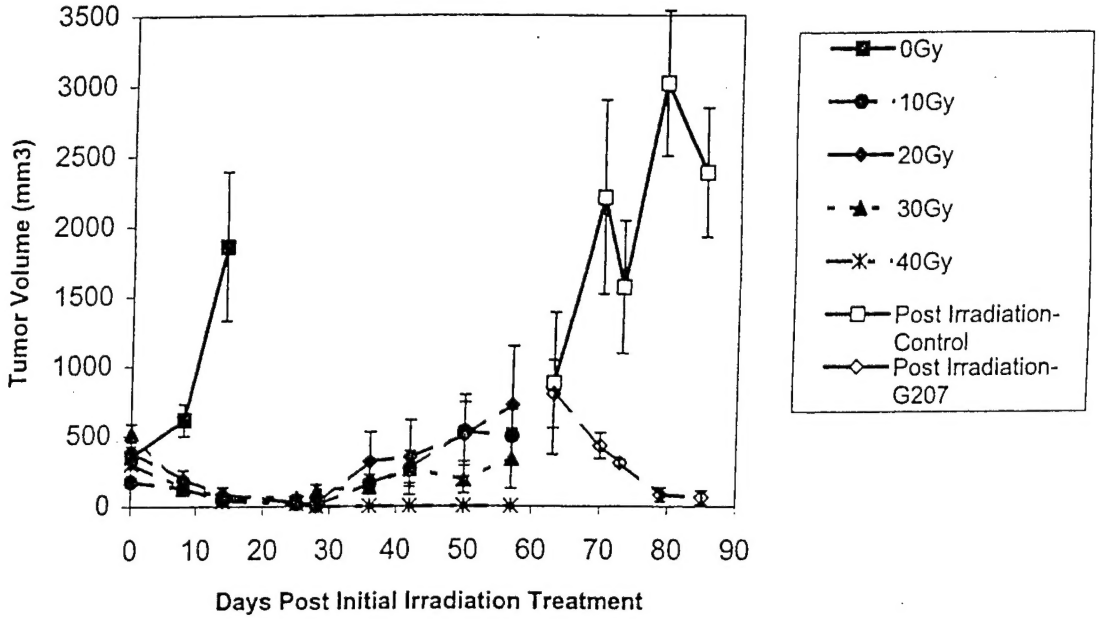
HYPOTHESIS 2: We expect to develop an in situ vaccine strategy for people with metastatic prostate cancer and to have results using one of the cytokine vectors by 24 months. As noted in the initial letter recommending funding (April 3, 1998) "This project was recommended for funding. The panel was intrigued with the possibility of using a mutated form of the herpes virus as a novel therapy for prostate cancer, but less enthusiastic about testing IL-12 and GM-CSF vectors." Therefore, in conjunction with the reduced funding of the grant, we have eliminated this aspect of the project as requested by the reviewers and are focusing on the other hypotheses. We shall attempt to obtain funding for this area of research through other means.

HYPOTHESIS 3. The G207 vectors will sensitize prostate cancer to radiation-induced cell death in vivo.

In the initial grant application, we noted that "although this concept of HSV sensitization of radiation-induced tumor cell killing has not been previously tested in prostate cancer, we have a reasonable expectation of success which could provide for the combination of these two technologies to be used in a synergistic fashion. We expect to have initial data on the interactions of G207 and radiation within 12 months".

We are doing studies to determine whether herpes viral gene therapies can be combined with radiotherapy of prostate tumors. We are using two animal models. One is the human xenograft model of LnCap tumor cells subcutaneously implanted in nude mice. Another is the TRAMP model, in which prostate tumors arising from transgenic C57 black mice are grown as cell lines in vitro and then implanted subcutaneously in syngeneic C57 black hosts.

In the LnCap model, we have found that the tumor is relatively sensitive to both the G207 virus and to the radiation. We have shown that tumors can be readily cured with either G207, or with 40 Gy of radiation fractionated over five consecutive days. We have also shown that for animals that fail radiation treatment, the locally recurrent tumors remain curative by viral therapy, suggesting that G207 remains a viable post-radiation treatment option for local recurrence. This is shown in the following figure.



The effect of radiation and G207 on subcutaneous LNCaP tumor growth. Subcutaneous LNCaP tumors were established in BALB/c *nu/nu* mice. When the mean tumor volume for LNCaP tumors (n = 15) was 348 mm^3 (range, 120–693 mm^3), tumors were randomized into five groups. Radiation was fractionated in equal doses over 5 days for a total dose of 10, 20, 30, or 40 Gy. Radiation treatment was noted to cause an initial reduction in tumor volume at all treatment doses when compared with 0 Gy. Tumor eradication was noted in 100% of the 40 Gy-treated tumors, and in 33% of the 30 Gy-treated tumors. All of these animals were then excluded from further studies. After the initial reduction in volume, all tumors in the 10- and 20-Gy treatment groups showed an increase in tumor volume. These tumors (n = 6) were then randomized to receive either G207 (1×10^7 PFU) intraneoplastically, followed 2 days later by a second injection, or two injections of virus buffer. G207-treated tumors had a marked reduction in tumor volume ($p < 0.05$ versus control on day 86) and tumor eradication was noted in 60% of the G207-treated tumors. (Bars represent mean tumor volume ± the standard error of the mean.)

In the TRAMP model, we have shown that subcutaneous tumors are relatively resistant to both G207 and radiation. Higher doses of virus and radiation are needed to obtain responses comparable to LnCap. For the most part, the tumors are incurable with the LnCap treatment regimens. Because of its relative resistance to therapy, we have chosen TRAMP as the preferred model to address the question of whether G207 and radiation can act additively or synergistically against prostate cancer. We are currently treating subcutaneous TRAMP tumors with G207 and radiation concurrently over seven days and assessing the effect on tumor growth compared to viral or radiation therapy alone. Treatment parameters determined from these studies with subcutaneous tumors will then be used in experiments where spontaneously arising prostate tumors in TRAMP mice are treated -- a condition more closely resembling the human situation.

KEY RESEARCH ACCOMPLISHMENTS

- We have demonstrated that multiple human prostate cancer cell lines are exquisitely sensitive to cytolysis by G207.

- We have demonstrated that G207 can effectively treat two different human prostate cancers grown in athymic mice.

- We have demonstrated that G207 is effective on both hormone sensitive and on hormone refractory prostate cancers.

- We have demonstrated that G207 is effective in human prostate cancer that is recurrent after radiation therapy.

REPORTABLE OUTCOMES

- We have prepared an initial manuscript of our work which has been accepted for publication. When published, a copy will be submitted in the next annual report.

- We have applied for additional funding for related studies both from private agencies and from the Department of Defense aimed at developing a clinical trial of herpes vectors for the treatment of prostate cancer and at exploring other avenues of this therapy not supported by this grant.

CONCLUSIONS: Having demonstrated in vitro that multiple human prostate cancer cell lines are exquisitely sensitive to cytolysis by G207 and that, in vivo, G207 can cure both hormone sensitive and hormone refractory and post-irradiation recurrent human prostate cancer, we feel that we have set the stage for the use of herpes vectors as a form of therapy for prostate cancer. We next foresee obtaining the necessary toxicity data and presenting this to the FDA for consideration as a clinical trial for locally recurrent cancer. This will open this area of research for further development and testing of even more efficacious vectors by us, by industry, and by other scientists and possibly the eventual treatment of widely metastatic cancers as well.

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